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A Comprehensive Review on Bioethanol Production from Fruit Wastes

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ABSTRACT

Bioethanol is a green resource of energy which is a product made from fermenting biological components. Fruit waste has high sugar content that act as a crucial factor for producing bioethanol. Bioethanol gained a significant attention as a promising alternative energy roots to gasoline, offering a clean, renewable, and green combustible fuel option. Bioethanol is categorized as three generations. The first-generation bioethanol has made from food crops such as sugarcane and corn, the second-generation non-food lignocellulosic materials like forestry leftovers and agricultural wastes, and the third generation advanced bioethanol incorporate innovative technologies like algae-based bioethanol and genetic engineering. The earliest recorded use of bioethanol was in ancient civilizations, where it was used as an alcoholic beverage. However, its use as a fuel source gained prominence in the 20th century, driven by the need for alternative energy sources. Cellulose is produced from the lignocellulosic mass and then fermented for bioethanol production. Lower lignin content also highlights its potency. Bulk amount of fruit wastes are being disposed throughout the world. They take a long time to degrade and may lead to several environmental threats. Thus choosing fruit waste as a feedstock greater benefits. The producing process of bioethanol contains many steps starts from pretreatment, hydrolysis, fermentation, and ethanol recovery. A pretreatment method involves methods like biological, physical and chemical. Hydrolysis involves production of cellulose from the lignocellulosic biomass. Blending ethanol with gasoline has numerous benefits, including enhancing the number of octane, reducing hydrocarbon emissions, and raising burning capacity. A range of countries have embraced ethanol-blended fuels, varying from E85 (85% ethanol, 15% gasoline) to E10 (10% ethanol, 90% gasoline) with some modifications to vehicle engines.

The bioethanol sector is a central figure in the global transformation to green energy. For bioethanol to become a mainstream energy source, its price needs to be competitive with that of fossil fuels. Governments worldwide have introduced policies like taxes, farm subsidies, and fuel requirements to encourage the production & utilization of biofuels. However, the high cost of producing bioethanol, largely driven by raw material expenses (60–75% of total costs), remains a significant hurdle. Employing cheaper feedstocks, such as waste biomass, and improving production methods can help lower these costs. The profitability of production of bioethanol deviates from one to another region; for example, America and Brazil have different production costs due to their distinct agricultural systems and available resources. Fruit waste stands out as a potentially valuable and cost-effective raw material for bioethanol production because it holds significant amounts of natural sugars, cellulose, and hemicellulose. The Food and Agriculture Organization (FAO) stated that global fruit production attained 887 million metric tonnes in 2020, with China, India, and Brazil being the leading producers. The accumulation of fruit and vegetable waste is a significant issue, with almost 37% of total agricultural waste in Asia being attributed to fruit and vegetable waste This has guided researchers to explore substitute feed stocks and production approaches to reduce costs and increase efficiency. Global fruit production has increased by 59% from 2000 to 2021, reaching a cumulative output of 910 million tons. Massive fruit production and intake led to impactful fruit waste formation, with 41 million tons of fruit waste produced annually. A waste of fruit peel alone aids about 15% - 60% of the fruit waste and mostly junked fruit waste can cause health issue if not properly managed, necessitating a suitable fruit waste management. This review article mainly focuses on highlighting the benefits of using fruit wastes for bioethanol production and the steps involved in it.

Keywords: *Bioethanol, Fruit wastes, Hydrolysis, Fermentation, Saccharification*

1. INTRODUCTION

1.1 Importance of Bioethanol as a Renewable fuel

Bioethanol, an alternative energy source produced via a process of fermentation of biological materials, has arised as a hopeful alternative to liquid petroleum (Dandasena et al., 2023). Bioethanol has earned major focus as a promising alternative fuel to gasoline, offering a clean, renewable, and green combustible fuel option. Bioethanol, a renewable fuel produced from biomass and bioenergy crops, has been extensively researched as a potential replacement for gasoline in internal combustion engines. Its adoption offers several key benefits, including a decrease in greenhouse gas emissions, an improvement in air quality, and greater energy independence (Momayez et al., 2017). Bioethanol, a renewable fuel with a history stretching back to ancient fermented beverages in China around 7000 BCE, transitioned to a significant fuel source in the 20th century as the quest for alternative energy intensified (Dandasena et al., 2023). It is classified into three categories as generations: first-generation bioethanol, which utilize food crops like sugarcane and corn; second-generation bioethanol, obtained from non-food lignocellulosic materials like forestry waste and agricultural wastes; and third-generation bioethanol, which employs advanced technologies using algae and cyanobacteria as feedstocks. These progressive approaches are geared towards enhancing sustainability and minimizing conflict with food resources (wi et al., 2013). Lignocellulosic biomass, the raw material for biofuel production, needs a series of carefully orchestrated steps to unlock its energy potential. First, pretreatment plays a major role in dismantling the robust architecture of the cell walls of plant. This stage aims to liberate cellulose, the primary sugar source, by cracking down lignin & modifying the crystalline arrangement of cellulose itself (Mohanty et al., 2016). As a beneficial side effect, the hemicellulose component is also broken down into its constituent sugars during this phase, adding to the pool of fermentable material.next, hydrolysis takes center stage. Here, specialized enzymes, namely cellulases and hemicellulases, act as biological scissors, cleaving the cellulose and hemicellulose chains into simple sugar units. Glucose, sourced from cellulose, & xylose, originating from hemicellulose, are the key sugars produced, ready for the next transformation. The fermentation stage is where the magic of microbial metabolismboccurs. Microorganisms, most commonly yeast like Saccharomyces cerevisiae, consume the liberated sugars. In this anaerobic process, they convert the sugars into ethanol, the desired biofuel, and carbon dioxide as a by product. Scientists are continuously developing engineered yeast types that can able to efficiently ferment a greater variety of sugars, boosting the overall process efficiency. Finally, ethanol recovery focuses on isolating the produced ethanol from the fermentation broth. Techniques like distillation, which exploits the different boiling points of ethanol and water, are employed to concentrate the ethanol. Further dehydration steps ensure that the resulting ethanol meets the stringent quality standards for use as a biofuel (Mohanty et al., 2016).Bioethanol stands out as a fuel with several beneficial traits. Its significant oxygen content, around 35% by weight, facilitates a more thorough burning process, boosting efficiency and lowering hydrocarbon & carbon monoxide emissions. The high number of octane roughly 108 gives bioethanol good resistance to engine knocking, allowing for higher compression ratios and potentially better performance. Furthermore, its considerable latent heat of vaporization, about 0.91 MJ/kg, helps cool the incoming air, which can also contribute to improved engine operation. One key consideration, however, is bioethanol's lower energy density, approximately 21 MJ per liter, compared to gasoline. This might require adjustments to engine systems to ensure comparable power delivery (Niphadkar et al., 2018). Ethanol’s capacity to be used independently or mixed with conventional fuels like gasoline and diesel presents a versatile pathway towards more sustainable vehicular energy, often enhancing engine performance and diminishing harmful emissions when blended with gasoline (Mohammed et al., 2021). The global bioethanol sector is central to the transition towards sustainable energy. However, its widespread use hinges on its ability to compete economically with conventional fossil fuels. Governments globally are actively encouraging biofuel production and consumption through policies like tax breaks, farm subsidies, and mandated fuel blending.

Nevertheless, the economic feasibility of producing bioethanol faces hurdles. A substantial portion of the expenses, ranging from 40% to 75%, stems from the cost of raw materials, especially biomass feedstocks. To tackle this, the industry is investigating cheaper and more sustainable alternatives, including agricultural waste and other forms of waste biomass. Progress in production techniques also holds promise for lowering costs and boosting efficiency. It’s worth noting that the cost-effectiveness of bioethanol production differs across regions, shaped by factors like the accessibility of feedstocks, farming methods, and technological development. For example, Brazil’s usage of sugarcane as a main feedstock has ended in lower production costs compared to the United States, where corn is the primary source. These regional variations highlight the necessity of customized approaches to enhance the economic stability of bioethanol production worldwide(Garcia et al., 2020). Global ethanol production has reached 29.03 billion gallons, with the America and Brazil leading the way. However, the cost of production remains a significant hurdle, primarily because over 55% of expenses are attributed to raw materials. Researchers are exploring the chance to use cheaper feedstocks, such as lignocellulosic biomass and agri-food waste, to tackle this challenge. The production charge of bioethanol is heavily influenced by the raw material, with sugarcane and corn exist as the most common. A promising chance for cost reduction lies in the improved conversion of xylose to ethanol. The growing concerns over pollution from fossil fuel overconsumption, especially in urban areas, underscore the urgent need for sustainable energy alternatives. Overall, the bioethanol economy presents a significant opportunity to generate employment and boost local economies, as demonstrated by the 147,000 jobs created by the US ethanol industry in 2004. Notably, fruit

biomass from mango and banana waste offers a sustainable solution that not only aids in waste management but also helps decrease greenhouse gas emissions and combat climate shift. This rearrangement presents the information in a slightly different flow while keeping all the original ideas and maintaining original phrasing (Demirbaş et al., 2005). Recent research highlights the potential of lignocellulosic biomass, obtained from sources like agricultural residues and industrial waste, as a feedstock for bioethanol production. This approach offers several benefits, including waste valorization, reduced contest with food crops for land, and lower manufacturing costs (Germec & Turhan, 2018). Bioethanol's history as a fuel traces back to ancient times, with significant advancements in its production methods occurring throughout the 20th century (Barua et al., 2023).

1.2 Justification for using Fruit Waste as a Biomass Source

The increasing quantities of fruit and vegetable waste have led to significant environmental concerns, including greenhouse gas emissions, the spread of diseases, and various forms of pollution. Traditional disposal methods like incineration, animal feed, composting, and landfill dumping are proving inadequate to handle this growing problem (Jahid et al., 2018). A promising sustainable solution involves the valorization of FVWs into biofuels like bioethanol, biohydrogen, biodiesel, & biogas. Fruit waste, with its significant content of natural sugars, cellulose, & hemicellulose, is particularly captivating as a feedstock for bioethanol production. The Food and Agriculture Organization (FAO) reported that global fruit production reached 887 million metric tonnes in 2020, with China, India, and Brazil being the top producing nations (Hamelinck et al., 2005). Consequently, the accumulation of fruit and vegetable waste represents a substantial challenge, with approximately 37% of total agricultural waste in Asia (Kumar et al., 2020; Itelima et al., 2013).

Numerous research efforts have examined the viability of producing bioethanol from fruit residues like banana, plantain, and pineapple peels by utilizing a range of enzymes and microbial strains. These investigations have yielded encouraging outcomes, identifying optimal factors like specific pH levels and temperature ranges for both fermentation and enzymatic breakdown. Mango peels, for instance, have been shown to possess a high concentration of reducing sugars, going up to 40% (w/v), highlighting their potential as a viable substrate for ethanol generation. Citrus fruits, commonly consumed either fresh or as juice, generate substantial lignocellulosic waste that can be harnessed for bioethanol synthesis. Researches have specifically inquired into the use of peels of plantain, banana, and pineapple in simultaneous saccharification and fermentation (SSF) processes involving microorganisms like Saccharomyces cerevisiae and Aspergillus niger. Research findings suggest that a temperature of 30°C and pH level of 6 are optimal for fermenting banana peels. Further experimentation with varying yeast concentrations has demonstrated that increased yeast levels can effectively shorten fermentation duration. Additionally, fruits such as pineapple and orange, which are rich in glucose, have been found to yield high amounts of bioethanol(Hossain et al., 2019). Fermenting mango peels directly has been shown to produce a relatively modest ethanol concentration of 5.4% (v/v). However, the addition of nutritional supplements like, the extract of yeast, bran, peptone, and wheat significantly enhanced ethanol yield, increasing it to as much as 7.14% (w/v). Studies evaluating the chemical composition of banana & mango residues for ethanol generation have indicated that applying acid pretreatment diluted followed by enzymatic hydrolysis is an effective strategy. This method facilitates the cracking down of cellulose & hemicellulose into fermentable sugars, improving overall bioethanol output(Yousif & Abdulhay, 2017).Conventional food crops like corn, sugarcane, and sugar beets are inadequate for satisfying the growing global demand for bioethanol, as they are primarily allocated for food and animal feed. Consequently, cellulosic sources like agricultural residues are emerging as more suitable alternatives for bioethanol production The ongoing study successfully demonstrated the capability of generating reducing sugars from peels of pineapple, banana, and orange, highlighting the viability of using plant-based waste for bioethanol production. (Moneruzzaman et al., 2021).

Ethanol has emerged as a renewable bio-energy source, offering a cleaner alternative to fossil fuels for powering automotive engines. With rising global fuel demand driven by population growth and industrialization, the production of bioethanol from low-cost, plant-based waste materials has become increasingly important. Utilizing agricultural waste, like potato waste, molasses, banana waste, and food grain waste, can grant a sustainable solution to environmental, economic, and energy concerns(Mtashobya et al., 2025).

Research has shown that FVWs, rich in carbohydrates and biodegradable, can replace fossil fuels. Microbial hydrolysis of FVWs transforms sugars into biofuels under anaerobic conditions. These biofuels can be utilized for cooking, electricity, and energy production. Eventhough biofuel production from FVW offers numerous advantages, it has yet to reach industrial and commercial scale. Ongoing research worldwide aims to overcome the challenges and make biofuel production from FVW a viable and sustainable solution (Hossain et al., 2023).

1.3 Research Gap

Second generation biomass feedstock has gained more popularity and preference than other feedstock in the recent years. Fruit waste has gained more importance due to its lavish availability and intense sugar content. This also helps farmers to increase their economy (Moura et al., 2024).

Even though they have more benefits they have several disadvantages too. Production costs are high while comparing with

traditional fossil fuels. Also fermentation of the lignocellulosic mass can be impacted by microbial inhibitors. Also pre-treatments for lignocellulosic biomass can be challenging due to its complex structure. Currently several research are on process to minimize the drawbacks and increase the bioethanol yield(Wang et al.,2016)..

2. BIOMASS SELECTION - FRUIT WASTE AS FEEDSTOCK

2.1 Types of fruits and sugar contents

The composition of various fruits by percent composition of sugars is presented in Figure 1.

2.2 Chemical Composition

The Chemical composition of Bio-derived material is a key factor to determine its appropriateness and efficiency for bioethanol production. The major components of biomass of interest for production of bioethanol are carbohydrates in the guise of simple sugars, starch, cellulose, & hemicellulose, and lignin, which is typically regarded as a drawback during enzymatic hydrolysis owing to its recalcitrant nature (Singh et al., 2015).

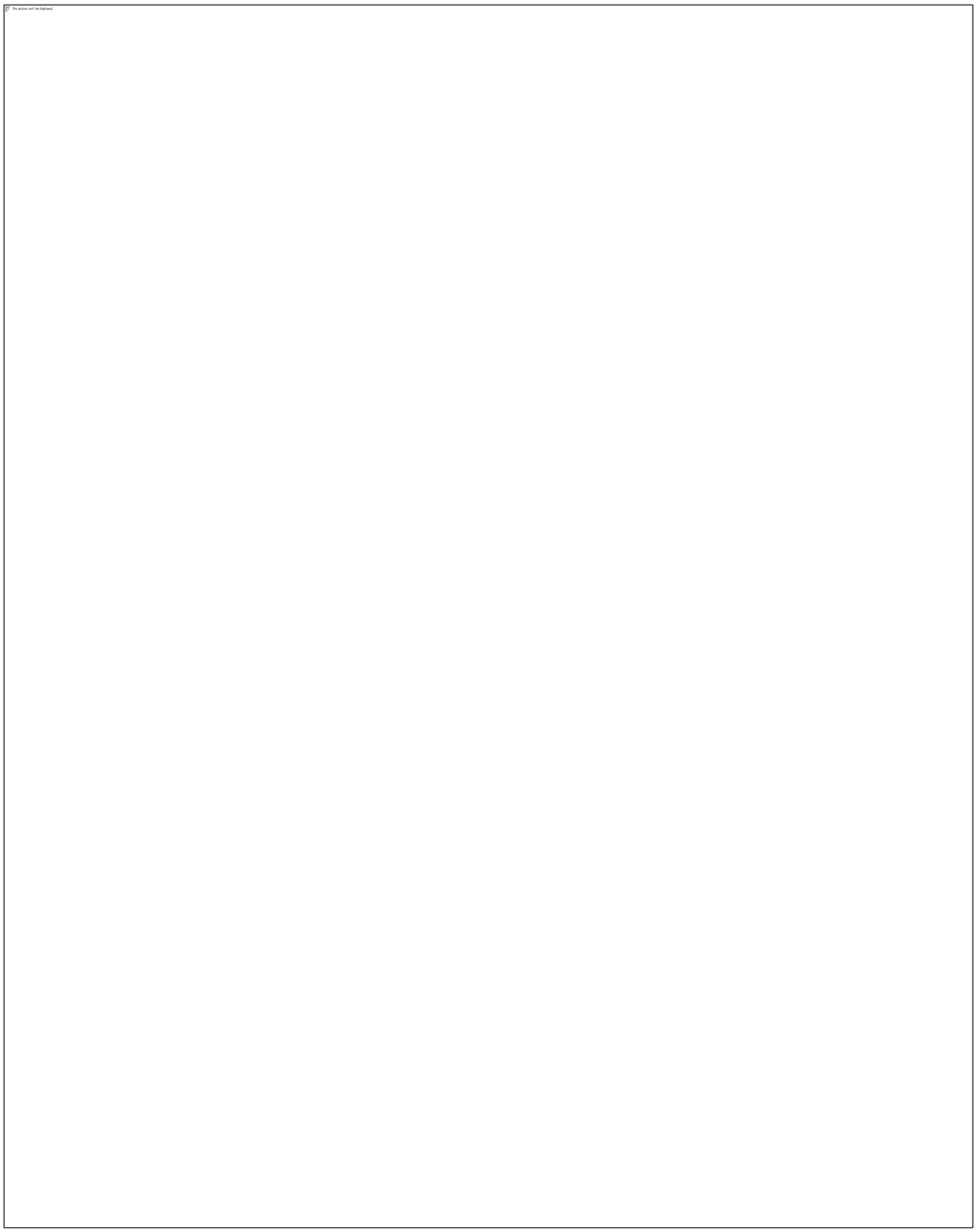

Figure 1 Fruits by percent composition of sugars

2.2.1 Sugars and Starch

Simple sugars (sucrose, fructose, glucose) and starch are the most easily fermentable carbohydrates found in fruit waste. These are generally hydrolysed readily and act as direct substrates for microorganisms that produce ethanol. High reducing sugar and starch contents have been found in banana peels and jackfruit waste, which can be used as feedstocks for production of bioethanol(Li et al., 2018).

2.2.2 Lignin

Lignin is an aromatic intricate polymer that gives cell walls of plants a stiffness and acts as a barrier to enzymatic hydrolysis. It strongly interacts with cellulose and hemicellulose, rendering the biomass recalcitrant. Lignin rich fruits, as in the case of jackfruit peel and grape stems, decreases the effectiveness of enzymatic hydrolysis and necessitates pretreatment techniques to enhance enzyme accessibility. It's a big and complicated molecule made up of three main building blocks called monomers: p- Coumarin, regular alcohols, and coniferyl. These monomers form an integrated and highly interlinked structure, contributing to its hardness and resistance to degradation. Unlike cellulose and hemicellulose, lignin lacks a sugar-based structure and instead features a 3D structure with alkyl-aryl bonds that act as an adhesive between cellulose & hemicellulose. [Chelgani, S. C., et. al., 2011]

The existence of lignin can appreciably reduce the performance of enzymatic hydrolysis via binding enzymes through, hydrogen bond, hydrophobic, & electrostatic interactions. Additionally, soluble lignin-derived chemical compounds can function enzyme inhibitors. (Chelgani et al., 2011)

2.2.3 Cellulose and Hemicellulose

Cellulose is a glucose monomer-based polysaccharide with β-1,4-glycosidic bonds. Cellulose makes up a considerable percentage of lignocellulosic biomass and needs to be enzymatically broken down into fermentable sugars. Hemicellulose is a pentose-hexose heterogeneous polymer, which also has a contribution towards the sugar content but is less resistant to hydrolysis than cellulose. Grapes, jackfruit skin, and banana peels all have different amounts of cellulose and hemicellulose. (Masri et al., 2010)

2.2.4 Lignocellulose

Lignocellulose biomass has a complex structure that contains both fermentable and non-fermentable sugars. Cellulose, the most common ingredient accounts for 33-47% of the biomass and is utilized for hydrolysis. Hemicellulose makes up 19-27% of the biomass. Unlike other components, lignin (5-24%) and silica (18.3%) are not fermentable. They form a complex that inhibits hydrolysis by adhering to cellulose. This reduces the surface area available for enzymes to operate and inhibits breakdown. (Krogell et al., 2015)

2.2.5 Cellulose

The percentage of cellulose in a feedstock is highly important in determining whether or not it can be utilized to produce bioethanol. Starting materials with higher cellulose content are generally it is easier to break cellulose, making it easier to access bioethanol production down to fermentable sugar. Hemicellulose is a complex group of polysaccharides it consists of a wide variety of short, branched chains of sugar, including arabino Glucuronoxylan, glucuronoxylan and galactoses. It is a polymer made up of both Hexose sugars (D-glucose, D-mannose and D-galactose) and abdominal sugars (D- xylose and L-arabinoses), and acetylated sugars. Structure of hemicellulose random and contains 5 or 6 carbon sugars (Celis et al., 2013).

2.2.6 Hemicellulose

Hemicellulose is the second most abundant polymer which is located in the secondary cell wall of plants. Xylan is the primary form of hemicellulose in plant cell walls and is converted into xylose during hydrolysis. This process can also produce by-products such as acetic acid, which can inhibit microbial growth and ethanol fermentation. To minimize the formation of by-products, it is essential to control the temperature and retention time during hemicellulose degradation. Due to its branched-chain structure and low molecular weight, hemicellulose can be easily hydrolysed. (Soltani et al., 2019). The typical Composition of Selected Fruit Waste Biomass are shown in Table 1

Table 1 Typical Composition of Selected Fruit Waste Biomass

Biomass Type	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Starch (%)	Sugars (%)
Banana	6 – 10	6 – 8	12 – 18	20 - 25	15 - 22

Jackfruit	10 – 15	5 - 10	20 – 25	10 - 12	10 – 18
Grape	10 – 12	8 - 10	25 – 30	2 - 5	5 – 12

2.3 Comparison of fruit waste with other biomass

Biomass can be categorized into four generations. First generation ethanol uses sugar and starchy crops. They possess a threat to biodiversity. Thus, second-generation biomass was introduced. This includes lignocellulosic biomass. Currently, second generation biomass is mostly preferred(Krogell et al., 2015). Third generation biomass includes deriving ethanol from algae as they possess 25 to 60% of carbohydrates. They have several disadvantages like high harvesting and processing costs (Chaudhary et al., 2021). Fourth generation involves using non-food crops and modified microbes which requires high investment. (Rawat et al., 2011).

Mixed biomass, comprising two or more types of biomasses, offers several advantages. By combining different biomass kinds, the final bio-fuel product's parameters can be improved. This is done by increasing the unique chemical and mechanical properties of each biomass type, resulting in a more efficient and effective fuel(Rawat et al., 2011). The usage of mixed biomass allows for greater flexibility in production of fuel, allowing the utilization of a broader variety of rawmaterials and reducing sugars dependence on a single biomass type. This can lead to improved energy density, combustion efficiency, and emissions.

Global fruit production has increased by 59% from 2000 to 2021. Fruit waste has higher bioethanol yield, low lignin content and fewer pretreatment requirements while comparing with another biomass. Also, they are abundantly available and disposed as waste.(Krogell et al., 2015).

2.3.1 Waste Fruit Versus First-Generation Biomass

First-generation biomass like sugarcane and corn are high in fermentable sugars, and thus high in ethanol yield. They are ethically problematic as they compete with food crops and agricultural land (Krogell et al., 2015). Fruit waste like jackfruit, banana, and grapes residues is an agro-industrial waste product, providing a food-secure, low-cost source. These wastes contain high concentrations of carbohydrates, particularly starches and sugars, making them an ideal substrate for enzymatic hydrolysis and fermentation

2.3.2 Fruit Waste Versus Lignocellulosic Biomass

Second-generation biomass like wheat straw, rice husk, & bagasse is structurally difficult and required to be pretreated to hydrolyse cellulose & hemicellulose into fermentable sugars. This adds process cost and energy requirement. Fruit waste has less lignin and is hydrolysis friendly, resulting in easier and energy-efficient conversion processes. (Chaudhary et al., 2021).

2.3.3 Fruit Waste Versus Algal Biomass

Algal biomass is highly productive and non-competitive with food crops. Nevertheless, its harvesting and cultivation processes remain technologically underdeveloped and costly. In contrast, fruit waste is easily obtained from markets, households, and processing industries, minimizing collection and processing costs. (Liu et al., 2021).

2.3.4 Environmental and Economic Considerations

Application of fruit waste to produce bioethanol also tackles the major area of solid waste management. Tons of fruit waste are produced worldwide, and improper disposal will cause environmental problems such as methane releases due to anaerobic breakdown in landfills. Production of leachate and attraction of vectors (Padmi et al., 2018).The issues and advantages of fruit wastes between different feedstock are shown in Table 2.

Table 2 Issues and advantages of fruit wastes between different feedstock

Biomass Type	Major Issue	Fruit waste Advantages
Fourth generation	High investment and advanced technologies	Fruit waste offers a simpler near- team solution
Third generation	High harvesting and processing cost	Fruit waste is easy to collect and process

Second generation	High Pretreatment cost	Fruit waste needs mild Pretreatment
First generation	Ethical concerns, land competition	Fruit waste avoids food vs fuel conflict

3. PRETREATMENT METHODS FOR BIOETHANOL PRODUCTION

3.1 Enzymatic Hydrolysis versus Acid Hydrolysis

Pretreatment methods are used to crack down biomass into its component parts, making it more accessible for further processing. Hydrolysis, also known as saccharification, is the process of breaking down cellulose & hemicellulose into sugars, which can be fermented into bioethanol. This process converts them into simple sugars that can be intended to produce ethanol. These simple sugars include maltotriose, maltose, sucrose, glucose, and fructose, which make up 60-70% of the total dissolved solids. There are three main ways to achieve hydrolysis: using enzymes, alkaline solutions, or acid. Acid hydrolysis is the oldest method used to convert plant waste into ethanol(Rozenfelde et al., 2017).

3.1.1 Acid Hydrolysis:

There are two kinds of acid hydrolysis:

(i). Concentrated acid hydrolysis: This method uses a strong acid at a low pressure and temperature. It takes a longer time to complete the process.

(ii). Dilute acid hydrolysis: This method uses a weaker acid at a high pressure and temperature. It is a faster process that can be done continuously.

Dilute acid hydrolysis is a method used to digest hemicellulose & prepare cellulose for enzyme access. This process involves a two-stage hydrolysis, where hemicellulose is converted at a lower temperature due to its structural differences with cellulose. The process typically done in a continuous flow reactor at 215°C by using a 1% sulfuric acid solution. However, the sugar recovery efficacy is limited to around 50%. The main challenge in dilute acid hydrolysis is achieving glucose yield above 70% still keeping a high cellulose hydrolysis level and minimizing glucose decomposition.(Rajak et al., 2016).

In contrast, concentrated acid hydrolysis provides quick and comprehensive cellulose hydrolysis to glucose and hemicellulose sugars with minimal degradation. This method uses relatively subtle temperatures and pressures generated by forcing between vessels. The reaction time is shorter than in dilute acid hydrolysis. The concentrated acid process involves the use of 30-40% sulfuric acid by soaking and dewatering the solid residue for 50 minutes, followed by additional cellulose hydrolysis at 373 K (Rozenfelde et al., 2017). The concentrated acid hydrolysis has a advantage due its higher sugar recovery efficiency and significant cost reduction compared to dilute sulfuric acid processes.

3.1.2 Enzymatic Hydrolysis:

The enzymatic hydrolysis process involves breaks down of cellulose into fermentable sugars using enzymes. This process requires specific enzymes, such as endo-β-1,4-glucanases, β-glucosidases, and cellobiohydrolase, to break down cellulose. The most effective and promising method is Enzymatic hydrolysis due to its specificity, low temperature requirements, and minimal production of inhibitors (Rajak et al., 2016).

There are two ways to achieve enzymatic hydrolysis: using microorganisms that produce enzymes or using readily available enzymes. The latter is more broadly used and viable. However, the high cost of enzymes remains a significant challenge in commercial-scale, cost-effective ethanol production.

The transition of lignocellulosic biomass to fermentable carbohydrates relies on factors like the type of biomass and hydrolysis conditions. Several factors influence the yield of sugar during enzymatic hydrolysis, which can be grouped into two categories: substrate-related factors and enzymatic and process-related factors.

A combination of enzymes, including endoglucanases, β-glucosidases, exoglucanases and cellobiohydrolases, is generally used. Each enzyme plays a specific role in breaking down cellulose and hemicellulose molecules.

- Endoglucanases aimlessly attack cellulose chains to make shorter polysaccharides.
- Remove hemicelluloses.

Comparison between enzymatic and acid hydrolysis is shown in Table 3.

Table 3 Comparison between Enzymatic Hydrolysis and Acid Hydrolysis

Parameters	**Enzymatic Hydrolysis**	**Acid Hydrolysis**
Type of Catalyst	strong acids (like HCL or H_2SO_4)	biological enzymes (e.g., cellulase, amylase)
Operating Condition	high temperature and low/high pH (severe conditions)	mild temperature and near-neutral pH (tender conditions)
Reaction time	Fast process (usually hours)	Slower process (take up to 24–72 hours)
Sugar yield	Can provide high sugar yield but may degrade sugars if too aggressive.	High yield with low sugar degradation
Inhibitor Formation	Forms toxic metabolites like HMF furfural that restrict fermentation	Low or no development of inhibitors.
Cost	Lower initial chemical expense but costly downstream detoxification	More expensive enzyme cost but less post-treatment requirements.
Selectivity	Non-specific – able to degrade multiple biomass components	Highly specific – specific towards specific bonds (e.g., glycosidic linkages)
Equipment Requirement	Needs corrosion-resistant equipment	Less equipment intensive; less equipment wears and tear
Scalability	Scalable but with environmental issues	Scalable, but enzyme availability and cost are limiting factors

3.2 Role of Amylase and Other Enzymes in Hydrolysis

3.2.1 Amylases

Amylases comprise a family of enzymes that are responsible for breaking down starch in the form of more basic sugar molecules, such as glucose, maltose, and dextrin's. These enzymes play both natural digestive functions and industrial roles where starch has to be digested into fermentable sugars. Amylases are categorized based on their action sites and mechanisms into three broad categories: beta-amylase, which is an exo-acting enzyme; alpha-amylase; endo-acting enzyme which is an; and gamma-amylase, each of which performs distinct and critical roles in the degradation of starch. (Bharadwaj et al., 2018)

Enzymatic hydrolysis is a biochemical reaction that involves the use of specific enzymes to break down complex molecules into simpler ones. The process relies on various enzymes, each with distinct functions. Carbohydrates, such as amylase and cellulase, are responsible for decomposing complex sugars. Proteases, on the other hand, degrade proteins into amino acids. Additionally, lipases hydrolyse fats into fatty acids. This enzymatic process typically happens at moderate temperatures, vary from 30°C to 60°C, which helps maintain enzyme activity and ensures optimal reaction rates(Vernon-Carter et al., 2019).

The vital role of amylases is to catalyse the breakdown of starch into fermentable sugars. In nature, enzymatic activity has a

significant role to play in aiding metabolic activities and energy production within organisms. Amylases, in industrial usage, are also responsible for reducing polysaccharides into simple sugars that can be furthered fermented by using bacteria or yeast. This fermentation process finds various uses, such as but not restricted to ethanol production, food processing, detergent making, and textile treatment. (Xu et al., 2016) Amylases have a major role in the production of bioethanol process, especially when using starch-rich waste materials, including fruit peels and pulp. Amylases catalyze the conversion of starch present in these fruit waste products to fermentable sugars, mostly glucose, during the saccharification step. The sugars are then subsequently fermented by microorganisms, including Saccharomyces cerevisiae (also called yeast), which leads to the generation of ethanol. The effectiveness of amylase activity is critical since it improves the accessibility of fermentable sugars, raises the total yield of ethanol, and reduces the processing time related to bioethanol production from complex waste streams.(Bharadwaj et al., 2018).

3.2.2 Function in Production of Bioethanol:

In the fermentation process, especially from starch-rich or fruit waste biomass, amylases are employed in a two-step enzymatic hydrolysis process:

Liquefaction: α-Amylase is supplemented to hydrolyse starch into shorter chains (dextrin) under elevated temperature 85–90°C (Li et al., 2014).

Saccharification: Glucoamylase is supplemented to hydrolyse dextrin into glucose under light acidic conditions (pH 4.5–5.5) and moderate temperature 55–60°C (Bharadwaj et al., 2018). The resulting glucose-rich hydrolysate is subsequently fermented by yeast or other ethanologenic microorganisms to yield ethanol.

3.2.3 Applications of Amylase:

Amylases are particularly important enzymes used in many industries because they can turn starch into simpler sugars. In the food industries, amylases are used to break down starch into liquid. This happens when making syrups, baking bread, brewing beer, and clarifying juice. In the textile industry, alpha amylases are used to remove starch coatings from fabrics before they are dyed. This process helps make the fabric softer and better in quality(Kim et al., 2011).

Amylases are added to laundry and dishwashing detergents to help get rid of stains made from starch on clothes and dishes. This makes the cleaning process work better, even when the water is cooler. In the paper and pulp industry, amylases help change starch during the sizing and coating steps, which improves the quality and strength of the paper. (Kim et al., 2011)

In bioethanol production, amylases break down starchy materials, like fruit waste, into sugars that can be fermented. These sugars are then turned into ethanol using microbes. Amylases are important because they are used in many different ways, both in old methods and in new biotechnological processes (Dhital et al., 2015).

3.2.4 Disadvantages and limitations of Amylases

Amylase is classified as a hydrolytic enzyme that facilitates the enzymatic breakdown of complex carbohydrates, including starch and glycogen, into simpler sugars such as maltose, glucose, and dextrin's (Memon et al., 2017) This enzyme is crucial for both biological digestion processes and industrial applications related to starch processing (Li et al.,2014).

Amylases can be classified into three principal types based on specific site of action they have on the polysaccharide chain: alpha-amylase, which is an endo-acting enzyme that breaks internal α-1,4-glycosidic bonds; beta-amylase, which is an exo-acting enzyme that breaks sugars at the non-reducing ends; and gamma-amylase, which is capable of acting on both α-1,4 and α-1,6 linkages (Li et al.,2014). Various Enzymes used in hydrolysis and the source of organisms and their role in fruit waste are shown in Table 4.

Table 4 Various Enzymes and their role in Hydrolysis

Enzyme	**Target Biomolecule**	**Main Products**	**Source Organisms**	**Role in Fruit Waste Hydrolysis**
Amylase	Starch	Glucose, Maltose	*Aspergillus spp., Bacillus spp.,*	Converts fruit starch
Cellulose	Cellulose	Glucose	*Trichoderma Reesei Aspergillus niger.,*	Breaks down cellulose from fruit peels and pulp

β – Glucosidase	Cellobiose (from cellulose)	Glucose	*Aspergillus niger, T. ressei, Yeasts*	Converts cellobiose to glucose,
Xylanase	Xylan (a hemicellulose)	Xylose, Xylo-oligosaccharides	*Bacillus subtilis, Aspergillus spp.*	Hydrolysis hemicellulose from fruit cell walls.
Pectinase	Pectin	Galacturonic acid, Oligo sugars	*Aspergillus niger, Rhizopus spp.*	Breaks down pectin in fruit tissues.
Swollenin	Plant cell wall polymers	Disruption of structure	*Trichoderma reesei*	Non-hydrolytic protein
Laccase	Lignin	Phenolic oxidized products	*Phanerochaete chrysosporium (White – rot fungi)*	Degrades lignin
Protease	Proteins	Peptides, Amino acids	*Bacillus spp., Asp*	Breaks down proteins in biomass.

3.3 Pretreatment Efficiency in Breaking down Complex Sugars

Pre-treatment is an important step Fermentable sugar. Physical pretreatment methods such as mechanics and ultrasound and heat treatment destroys the physical structure of waste, growing its floor vicinity and accessibility to enzymatic or microbial movement (Gavrila et al., 2024; Kannah et al., 2021).

The performance of enzymatic hydrolysis in changing lignocellulosic biomass into fermentable sugars is impacted by way of diverse inhibitors. those inhibitors can be labelled into several agencies, including (Tiwari et al., 2017).

1. Sugars and degradation merchandise: Glucose, cellobiose, and other soluble sugars can inhibit enzyme hobby via binding to the lively sites of cellulases, lowering their efficiency. Additionally, degradation products like furan derivatives and phenolic compounds can also hinder enzymatic hydrolysis.

2. Lignin and pseudo-lignin: Lignin, a complex phenolic polymer, can act as a physical barrier or bind unproductively to enzymes, reducing their activity. Pseudo-lignin, formed during pretreatment, can also restrict enzymatic hydrolysis by non-productive adsorption.

3. Oxidized products: The action of lytic polysaccharide monooxygenases (LPMOs) can produce oxidized sugars, which may impact enzyme activity.

4. Water constraint and rheological properties: High-solids loadings can lead to water constraint, reducing enzyme diffusion and activity. The rheological properties of the slurry can also affect enzyme efficiency (Kannah et al., 2021; Demiray et al., 2019).

Pretreatment of lignocellulose biomass (LCB) is crucial for efficient of bioethanol production. Various pretreatment methods have been explored, including extrusion, steam explosion, and ionic liquids. However, challenges persist, such as energy intensity, inhibitor formation, environmental concerns, high costs. To overcome these challenges, research focuses on developing novel, more effective pretreatment methods, integrating multiple pretreatment techniques, utilizing nanomaterials as Nanocatalysts, optimizing existing pretreatment methods, exploring new biomass sources. The goal is to enhance biomass bio-accessibility, reduce costs, and minimize environmental impacts, ultimately making large-scale bioethanol production from LCB more practical and sustainable (Nargotra et al., 2020).

4. FERMENTATION PROCESS

4.1 Role of Yeasts in Fermentation

Yeasts are the crucial biocatalysts in the process of fermentation for bioethanol production, which transform fermentable sugars into ethanol and carbon dioxide via anaerobic metabolism. Of all types of yeast species, *Saccharomyces cerevisiae* is the one that has been commonly used because it is a strong ethanol producer with high fermentation rate and ethanol tolerance**.**

Saccharomyces cerevisiae has been the standard organism for industrial production of bioethanol beacause of:(Nargotra et al., 2020). This one has

•Supeior hexose conversion efficiency into ethanol.

•Resistance to ethanol concentration near 15% (v/v).

•Sturdy growth and ease of growth.

•Access to extensive metabolic and genetic manipulability tools for optimization.

Yet, one of the major limitations of *Saccharomyces cerevisiae* is that it is not capable of fermenting pentose sugars (e.g., arabinose, xylose), which are common in lignocellulosic and fruit waste hydrolysates (Wei Ye et al., 2016).

4.1.1 Other Yeasts Employed in Bioethanol Production

To overcome S. cerevisiae's shortcomings, other pentose-fermenting or stress-tolerant yeast species have been sought out.

Pichia stipitis (Schizosaccharomyces stipitis): Known for being able to ferment xylose very efficiently under low-oxygen conditions. It tends to be applied in co-culture fermentations or genetically modified systems (Tahir et al., 2022; Oguri et al., 2011).

Candida shehatae: Ferment glucose and xylose but are less ethanol-tolerant than S. cerevisiae. Can be used for microaerobic fermentation.(Fromanger et al., 2010)

Kluyveromyces marxianus: A thermotolerant yeast with a wide sugar fermentation ability and capability to operate at increased temperatures (up to 45°C), making it a good choice for simultaneous saccharification and fermentation (Tahir et al., 2022, Goshima., et al., 2013 & Sukhang et al., 2020).

Zygosaccharomyces bailli: Tolerates inhibitory compounds and the low pH frequently found in fruit waste hydrolysates. . (Stessl et al., 2020 & Yan Xu et al., 2017)

4.1.2 Mixed Cultures and Genetic Engineering Approaches

Current research has been aimed at the utilization of mixed yeast cultures and genetically modified strains to increase fermentation efficiency and expand substrate range. Co-cultivation of S. cerevisiae with pentose-fermenting yeasts facilitates the conversion of mixed sugar substrates, enhancing overall ethanol yield (Sukhang et al., 2020).

Genetic modification of S. cerevisiae has also made it possible to introduce xylose and arabinose fermentation pathways, further increasing its application in lignocellulosic and fruit waste bioethanol production (Wei Ye et al., 2016).

4.2 Fermentation condition

Fermentation is a key stage in the bioethanol manufacturing process, wherein microorganisms like yeast or bacteria break down sugars from biomass into ethanol. Optimizing a number of fermentation conditions like temperature, pH, inoculum concentration, fermentation time, and substrate concentration plays a crucial role in ensuring the efficacy of the biochemical conversion. All of these conditions have a direct impact on the cellular processes of the microorganisms and, ultimately, on ethanol production. (Xu et al., 2017; Tenkolu et al., 2024)

4.2.1 Temperature

Temperature affects biochemical pathways by deactivating enzyme structure. Saccharification is typically attained at 95-105 °C, while fermentation temperature varies from 25 to 30 °C. The temperature of fermentation has a great influence on the and metabolic process of the fermenting microorganisms. The ideal temperature for Saccharomyces cerevisiae, the most popular yeast, is between 30°C and 35°C. Temperatures outside this range may result in thermal inactivation of enzymes and denaturation of microbial cells, lowering ethanol production. (Laluce et al., 2009; King et al., 1982)

4.2.2 pH

It is necessary to maintain a suitable pH for the activity of microbes and enzyme. The most suitable pH for ethanol fermentation by S. cerevisiae vary from 4.0 to 5.0. A low pH inhibits the growth of unwanted microbes while sustaining yeast activity. Highly acidic or basic pH levels may inhibit microbial growth and decrease fermentation efficiency. pH can alter the nature of proteins and affect enzyme activity. A very low pH can denature enzymes, while a higher pH can lead to

acid production instead of bioethanol. (King et al., 1982; Okoye et al., 2017)

4.2.3 Inoculum Size

The size of yeast or microbial culture added to the fermentation medium, referred to as inoculum size, influences the rate and degree of fermentation. An increased inoculum can decrease the lag phase and enhance the rate of ethanol production, but high inoculum can result in nutrient exhaustion and more biomass than ethanol.The size of the initial inoculum affects microbial cell density during fermentation. A small inoculum size can lead to a longer lag phase, while a large inoculum can cause substrate competition. (Laluce et al., 2009; Okoye et al., 2017)

4.2.4 Fermentation Time

The length of fermentation affects the final concentration of ethanol. Typically, 48–72 hours of fermentation is adequate for optimal production of ethanol, relying on the substrate and microorganism employed. Extended fermentation can be result in ethanol oxidation and yield reduction.Fermentation productivity is influenced by incubation time. Extended fermentation time can lead to more energy usage and increased production costs (Tenkolu et al., 2024).

4.2.5 Substrate Concentration

The sugar level in the fermentation medium should be optimized to prevent substrate inhibition. High sugar concentrations may cause osmotic stress to microbial cells, whereas too low levels might not offer enough carbon sources for effective ethanol production (King et al., 1982 & Ratnam et al., 2005).

4.2.6 Oxygen Supply

While ethanol fermentation is an anaerobic condition, a little oxygen at the beginning can stimulate yeast development. However, extended aeration directs metabolism to cell biomass formation instead of ethanol, hence lowering efficiency (Okoye et al., 2017).

4.2.7 Nutrient Availability

Micronutrients like nitrogen, phosphorus, magnesium, and vitamins are required for microbial metabolism and the process of ethanol synthesis. Addition of required nutrients to the fermentation medium can be used for maximizing ethanol yield and fermentation rate. Optimization of such fermentation conditions is critical, particularly with the use of fruit waste mixed as a substrate, as its composition is variable. The knowledge of the interaction between these parameters helps one formulate effective fermentation strategies, leading to increased ethanol productivity and cost-effectiveness of the process of bioethanol production.(Ratnam et al., 2005)

4.2.8 Anaerobic Conditions

Bioethanol fermentation is primarily anaerobic, since oxygen restricts ethanol production by re-routing metabolism into biomass production instead of ethanol. Sustaining rigorous anaerobic conditions once primary yeast growth has occurred is important. (Okoye et al., 2017)

4.2.9 Yeast (Inoculum) Concentration

Yeast level has a considerable influence on the rate and quantity of fermentation. A suitable inoculum (usually 5–10% v/v) provides instant utilization of sugars and ethanol formation. Insufficient yeast retards fermentation: excess can exhaust nutrients and add by-products. (Laluce et al., 2009)

4.2.10 Moisture Content

Moisture affects microbial metabolism, nutrient solubility, and heat exchange. For solid-state or semi-solid fermentation (which is typical in fruit waste systems), optimal moisture (60–70%) provides accessibility to the substrate and yeast viability. High moisture content can dilute sugar concentration, whereas low moisture can inhibit microbial metabolism.

Such fermentation conditions can be optimized, especially with the utilization of mixed fruit wastes as a substrate, because they contain variable composition. The interaction of such parameters makes it easy to design efficient fermentation strategies, resulting in enhanced ethanol productivity and cost-saving in the bioethanol process.

4.3 Aerobic and Anaerobic Fermentation

The fermentation is a pivotal process in the bioethanol producing process, where the microbial conversion of sugars to ethanol takes place. Based on whether oxygen is present or not, fermentation can either be aerobic or anaerobic. The form of fermentation utilized acts a great role in the efficiency and output of ethanol manufacture (Kuriyama et al., 1993).

Aerobic fermentation is performed with the presence of oxygen. In this treatment, microorganisms like Saccharomyces cerevisiae grow sugars mostly towards biomass instead of ethanol production. The yield in energy (formation of ATP) is greater aerobically but the ethanol production is low from the perspective that sugars are fermented into carbon dioxide and water as opposed to ethanol. Though aerobic process helps to grow the yeast cells at a faster level and also add vigoro, under

most cases they are not better suited for accumulation of ethanol.(Oliveira et al., 2024)

Anaerobic fermentation, which takes place without oxygen, is the industrially favoured route for bioethanol production. Anaerobic fermentation sees yeasts reduce sugars like glucose, fructose, and sucrose into ethanol and carbon dioxide. The pathway is less energy-efficient for the microorganism but yields much greater amounts of ethanol. Anaerobic fermentation is particularly crucial when dealing with fruit waste, which has fermentable sugars and is frequently water-rich, making it easy to create the anaerobic condition.(Berlowska et al., 2017)

In the bioethanol production from mixed fruit waste such as jackfruit, banana, apple, and grapes, anaerobic fermentation is the usual method because it has maximum ethanol yield and effectiveness in the process. The natural sugar content of fruit waste makes it very good for direct anaerobic fermentation, particularly if it is followed by enzymatic hydrolysis to release fermentable sugars (De silva et al., 2022). The Comparison between Aerobic and Anaerobic Fermentation shown in Table 5..

Table 5 Comparison between Aerobic and Anaerobic Fermentation

Characteristics	**Aerobic Fermentation**	**Anaerobic Fermentation**
Oxygen Requirement	Required	Not required
Energy yield	High	Low
Application in Bioethanol	Limited	Preferred method
Principal products	CO2, water, biomass	Ethanol, CO2
Ethanol yield	Low	High

4.4 Factors Affecting Ethanol Production

The ethanol yield from fruit waste biomass is greatly affected by a number of factors, especially when enzymatic hydrolysis is followed by fermentation. It is important to know these parameters in order to increase the conversion efficiency of sugars to ethanol.

4.4.1 Biomass Type and Composition

Various fruit wastes (jackfruit, banana, grapes) differ in their carbohydrate content, moisture content, fibre structure, and natural inhibitors. Peels of jackfruit and banana are high in starch and cellulose, whereas grapes have higher simple sugar content. These variations affect the level of enzymic hydrolysis, and the fermentable sugars produced. (Palacios, et al., 2017)

4.4.2 Pre-treatment Techniques

Pre-treatment degrades the lignocellulosic complex structure of the biomass, making enzymes more accessible. Typical techniques involve physical (milling), chemical (acid or alkali), or biological (microbial action). Efficient pre-treatment improves enzymatic efficiency and, therefore, ethanol yield. (Weiss et al., 2019)

4.4.3 Enzyme Type and Dosage

Amylases, cellulases, and pectinases play key roles in the hydrolysis of polysaccharides into simple sugars. The extent and nature of the enzyme applied influence hydrolysis efficacy. Inadequate dosage leads to partial hydrolysis, whereas the employment of too much enzyme is uneconomic unless yield gain is substantial. (Bayitse et al., 2015)

4.4.4 pH and Temperature

Enzymatic activity is extremely sensitive to conditions. The majority of hydrolytic enzymes act best in a particular pH (typically 4.5–6.0) and temperature range (30 60°C). Departures from these conditions can denature the enzyme or decrease catalytic efficiency and hence reduce sugar release and ethanol yield. (Hawaz et al., 2024)

4.4.5 Fermentation Condition

Ethanol production is also affected by fermentation conditions, such as:

- Yeast strain: Saccharomyces cerevisiae is typically employed due to its high tolerance to ethanol.

- Inoculum size: Adequate yeast concentration is required for vigorous fermentation.
- Oxygen levels: Anaerobic conditions are needed for ethanol production.
- Fermentation time and temperature: Longer time and ideal temperature (usually around 30°C) optimize ethanol conversion. (Altınısık et al., 2024)

4.4.6 Inhibitor Formation

During pre-treatment, furfural, Hydroxymethylfurfural (HMF), and phenolics can occur and inhibit enzymatic activity and microbial fermentation. Detoxification processes or tolerant yeast strains can be necessary to overcome this problem (Vanmarcke et al., 2021).

4.4.7 Solid Loading and Moisture Content

The solid-to-liquid ratio influences enzyme-substrate interactions. Solid loading can enhance ethanol concentration but suppress enzyme activity because of substrate inhibition or mixing issues (Bayitse et al., 2015).

5. OPTIMIZATION OF PARAMETERS

The significance of parameters for bioethanol production by fermentation process is crucial for maximizing bioethanol yield from fruit waste. Investigating and fine-tuning factors such as temperature, acidity levels (pH), the amount of yeast used, and the duration of fermentation significantly improves how well sugars are transformed into ethanol, thereby boosting overall output. These efforts aid in pinpointing the most favorable settings for sugar conversion and alcohol creation. Keeping a close watch on these factors helps to avoid the formation of undesirable substances and contamination. This ensures the consistency, repeatability, and potential for scaling up the process. Optimization leads to less time spent, reduced energy consumption, and less waste of resources. Ultimately, a thorough understanding of these parameters results in a more streamlined and economical fermentation procedure. By carefully examining these conditions, we can establish an optimal environment for achieving the highest possible ethanol yield.

Employing Response Surface Methodology (RSM) and Design of Experiments (DoE) presents considerable benefits when it comes to fine-tuning process parameters. These approaches allow for more dependable findings with a reduced number of experiments, generate more comprehensive data, and facilitate the investigation of how various factors interact to optimize processes (celis et al., 2013). Optimization driven by RSM has led to significant progress in increasing bioethanol production from sugarcane molasses.

Considering the multitude of factors that have an impact and the economic aspects at each step of bioethanol production pretreatment, enzymatic breakdown, and fermentation, Design of Experiments (DoE) and Response Surface Methodology (RSM) have become increasingly crucial for achieving optimal results. These methodologies play a key role in developing practical and efficient methods for transforming lignocellulosic biomass into bioethanol, offering economically sustainable industrial applications (chelgani et al., 2011; krogell et al., 2015; Chaudhary et al., 2021; Liu et al., 2021). Response surface optimization stands out as a highly regarded and commonly used techniques for structuring experiments and performing data analysis within the field of microbial culture. When compared to examining one factor at a time and using orthogonal tests, this method provides a more complete picture, substantially decreases the batch of experiments needed, and recognizes the best fermentation conditions using minimum experimental information. This not just reduces the amount of work engaged in optimizing fermentation but also upgrades the precision and logical basis of the whole process (Rawat et al., 2011).

5.1 Studies of pH, Yeast Concentration, Temperature, Fermentation Time

5.1.1 Effect of pH on Ethanol Yield

The acidity of the fermentation medium is a key factor of yeast metabolism and ethanol productivity. Studies have investigated the optimum range of pH of fermentation media which affects the yeast growth and ethanol yield, by using diverse substrates. For instance, Arif et al. (2018) evaluated bioethanol production from jackfruit seed hydrolysate under separate hydrolysis fermentation (SHF) at 30 °c for 72h, estimated that pH 3.0 yielded the highest ethanol concentration of 57.94%(v/v) compared to pH 2.0, 4.0, and 5.0 (Dandasena et al.2023). In another study, Sandesh Babu et al., (2014) estimated four fruit juices fermented by S. cerevisiae at initial pH values varies from 4.0 to 6.0, finding pH 5.0 optimal with sugarcane juice producing 520µg/ml ethanol after 48h (Momayez et al., 2017).De silva et.al., (2022) further confirmed pH 5.0 as broadly effective for banana and grape waste fermentations at 30 °c yielding 39.46g/L (5.11% v/v) and 46.77 g/L (6.08% v/v) ethanol, respectively (Srivastava et al., 2022). Overall, these studies showed that the optimal pH range that maximizes ethanol yield is between 3.0-5.5 depending on substrate and yeast strain.

In summary, the slightly acidic medium at pH 5 has been proven optimal for ethanol production by choosen S. cerevisiae strains in both monoculture and coculturcouture fermentations achieving maximum productivity (Mohammed et al., 2021). At higher pH values (>6), ethanol yield and sugar conversion rates declines, due to redirection of carbon flux toward glycerol and organic acid byproducts such as acetate (Niphadkar et al., 2018). Conversely, for seed-based substrates rich in complex

carbohydrates such as jackfruit seed hydrolysate lower pH range around 3.0 favor hydrolytic enzyme activity and yeast performance, yielding up to 57.94 % (v/v) ethanol under SHF conditions (wi et al., 2013). Sugar-rich fruit wastes (e.g., overripe banana and grape) demonstrate optimal fermentations within the pH range of 4.5–5.5, with ethanol concentrations (Srivastava et al., 2022). Finally, lignocellulosic hydrolysates derived from empty fruit bunches, rice straw, and corn stover exhibit peak fermentative efficiencies at pH 4.5–5.5 balancing with enzymatic saccharification optima and facilitating complete sugar utilization for ethanol synthesis (wi et al.,2015).

5.2 Yeast Concentration and Factors Affecting Yeast Growth

Bioethanol production controls urasyeast ability to ferment six-carbon sugars (e.g., glucose) into bioethanol and carbon dioxide via alcoholic and glycolysis fermentation bypassing complete oxidation in the tricarboxylic acid cycle. Notably, Crabtree - positive yeasts such as Saccharomyces cerevisiae will continue to produce and buildup ethanol even under aerobic conditions, a phenomenon termed the Crabtree effect (Garcia et al., 2020; Micic & Jotanovic 2015; Demirbas, 2005).

For countless generations, microorganisms like *Saccharomyces cerevisiae* have been applied in the creation of alcoholic beverages, most notably in the beer and wine sectors. Its capacity to generate a significant amount of ethanol, coupled with its rapid rate of production and ability to endure high levels of alcohol, helps to minimize the expenses associated with distillation(Germec & Turhan, 2018). Growth and metabolic rates of Saccharomyces cerevisiae rise with increasing temperature up to an optimum range of app~ 25–35 °C, beyond this range cellular performance declines.Ethanol accumulation around 40 g L^{-1} disrupts membrane integrity and key metabolic pathways, gradually reducing yeast viability (Itelima et al., 2013). While S.cerevisiae ferments hexose sugars efficiently, only species of the genera Pichia, Candida, Schizosaccharomyces and Pachysolen can convert pentose sugars into ethanol (Hossain et al., 2019). S. cerevisiae can thrive at pH values between 4.0 and 6.0, the optimal value of pH for ethanol production is about 5.0–5.5. A highest ethanol level of 36.85g/L, productivity of 3.07g/L·h, and 93.61% yield were achieved by fermenting PWH with S. cerevisiae HG1.1 at 40°C, pH 5.5, using 8.0x10^7cells/mL yeast and 4.95 g/L yeast extract(Yousif & Abdulhay, 2017). The quantity of the yeast inoculum significantly affects fermentation of fruit-waste. While small amount of yeast slows fermentation and increases contamination risk, and too much amount, wastes resources and oxygen during yeast growth(Hossain al., 2019). Weldehans and Halefom (2015) found that just 0.10 % S. cerevisiae gave near-maximum ethanol from banana waste using optimized conditions (30-40°C, pH 4.5, 48h), predicting around 0.48 g/L for pulp and 0.46 g/L for peel(Khandaker et al., 2020).

Nwogwugwu et al. (2021) estimated that a 10 % v/v yeast inoculum of Cronobacter malonaticus was ideal for ethanol production from Calabash pulp by solid-state fermentation under a condition of pH of 6.08 and a temperature of 28°C which gave a ethanol yield of 5.08 % v/v (Gireesh & Priya, 2020). Hawaz et al. (2023) found that a 20 % v/v of yeast concentration yielded 86% of (56 g L^{-1}) from sugarcane molasses. Even though molasses differs from fruit waste, the inoculum sizes between 10–20 % v/v are frequently optimal for maximizing ethanol production (Raagapriya et al., 2016).

Increasing the *Meyerozyma caribbica* MJTm3 inoculum in sugarcane molasses fermentation from 15 % to 20 % v/v boosted alcohol content from 14.6 % v/v to 95 % of the theoretical yield, highlighting that the ideal inoculum size within the 10-20 % range can vary depending on the specific yeast strain (Mtashobya et al., 2025). For *Meyerozyma caribbica* MJTm3 fermenting sugarcane molasses, raising the inoculum volume from 15 % to 20 % v/v increased alcohol production from 14.6 % v/v to 95 % of the theoretical maximum. This suggests that the ideal amount of starting yeast culture, within the 10-20% bracket, is unique to the particular type of yeast being used (Raagapriya et al., 2016). Generally, choosing the right amount of yeast to start with is vital for effectively producing ethanol from waste materials derived from fruits. For instance, very small amounts of yeast (0.10% weight/volume) can result in approximately 0.48 grams of ethanol per liter when using liquid extracted from banana peels, provided the acidity and temperature are properly adjusted. Meanwhile, moderate amounts of yeast (10% volume/volume) work well for both solid and liquid fermentations of Calabash pulp, yielding between 5 and 9 grams of ethanol per liter. Conversely, high inocula (15–20 % v/v) are advantageous for stress-tolerant or mixed-sugar substrates such as Meyerozyma caribbica fermentations of sugarcane molasses yielding up to 56 g L^{-1} ethanol and >90 % of theoretical conversion. These data-driven benchmarks, derived via response surface methodology, provide a practical framework for scaling bioethanol processes across diverse fruit waste streams(Khandaker et al., 2020; Gireesh & Priya, 2020; Raagapriya et al., 2016).

5.3 Optimal Temparature and Time for Fermentation Media

Optimization of temperature and time period for fermentation media is crucial for maximizing ethanol yield from various fruit wastes. Maintaining both temperature and time under the suitable condition depending on the kind of substrate, the fermentation process ensures efficient sugar conversion and result in high process scalability (Raagapriya et al., 2016; Mtashobya et al., 2025). Salihu U.Y. et al., (2022) experimented the different temperature conditions of 25°c. to 45°C at pH 4.0 for 72h for fermenting sugarcane molasses, resulted in 77%of yield at temperature 35°c which is ideal compared to other temperature(Hossain, 2023).

As same, Phong et al., (2022) estimated a 96-hour fermentation study of Cronobacter malonaticus at 25 °C, 32.5 °C, and 40 °C, while sampling every 24 h, found that optimal ethanol production (5.08 % v/v on day 3) between 28 °C and 32 °C (pH

6.08, 10 % v/v inoculum) according to RSM (desirability 0.911). Over the 96 hours, reducing sugars decreased (3.50 to 2.87 g/L), while cell density (OD_{600}) peaked at 0.6607 on day 3 before a slight decline(Wang et al., 2016). Studies on mixed fruit wastes (kinnow peels + banana peels) found that a fermentation temperature of 30 °C provided the highest ethanol yield (26.84 g L^{-1}) and efficiency (83.5%) when 6% (v/v) S. cerevisiae G and 4% (v/v) Pachysolen tannophilus MTCC 1077 were co-inoculated after steam explosion and enzymatic saccharification. Below 25 °C, sugar uptake slowed, and above 35 °C, yeast viability declined due to heat stress (Khandaker et al., 2020). In submerged SSF of banana peels, temperature was varied from 20 °C to 50 °C at fixed pH 6 and 4% (v/v) A. niger + 3% (w/v) S. cerevisiae. Maximum ethanol yield (6.29% v/v) occurred at 30 °C; at 20 °C, yield dropped by 60%, while at 40 °C and above viability and ethanol productivity fell sharply. This study underscores the narrow optimal window around 30 °C for banana peels (Li et al., 2018).

Apple pomace solid-state fermentations likewise center on 30 °C: hydrothermal + enzymatic pretreatment followed by SSF with S. cerevisiae Y51 gave the best yield (5.23% v/v) at 30 °C and 55 rpm agitation; trials at 25 °C and 40 °C yielded 20–30% less ethanol, attributed to suboptimal enzyme activity and yeast metabolism outside the 30 °C sweet spot (Soltani,2019). Notably, even within apple pomace systems, slight deviations (±2 °C) from 30 °C can cut yield by 5–10%.

Industrial-scale hydrolysate fermentations of apple pomace also adopt ~30 °C. At Michigan State University's pilot plant, dilute-acid and CaO-pretreated apple pomace hydrolysates fermented at 30 °C yielded 38.8 g L^{-1} and 36.9 g L^{-1} ethanol, respectively, with unhydrolyzed solids causing minimal inhibition.Trials at 25 °C slowed sugar consumption by >40%; at 35 °C, ethanol productivity rose slightly but cell lysis increased, making 30 °C the preferred compromise (Masri et al., 2010).

However, some fruit wastes benefit from mildly higher temperatures. Papaya peel hydrolysate fermentations showed maximal ethanol concentration (0.224 g mL^{-1}) at 45 °C and 24 h, pH 4.5, attributed to enhanced thermotolerance of a mixed-culture yeast consortium and faster sugar diffusion at higher temperatures (Demirbaş, 2005). Yet, this is an exception most studies on fruit peels and pomace converge on 30 °C as optimal, reflecting the native temperature preference of S. cerevisiae and many co-cultured fungi.

In summary, across diverse fruit wastes banana peels, kinnow peels, apple pomace, and others the optimal fermentation temperature for ethanol production by S. cerevisiae (often in co-culture) falls in the 30 °C ± 2 °C range (Khandaker et al., 2020; Li et al., 2018; Soltani, ,2019; Masri et al., 2010), with rare cases (e.g., papaya peels) showing higher optima when using thermotolerant strains or mixed cultures (Demirbaş, 2005). Operating at this temperature balances yeast growth, enzyme activity, and ethanol toxicity to maximize yield and productivity.

5.4 Findings from Previous Research on Optimal Conditions for Yield

Most of the studies has applied statistical designs, RSM and CCD to optimize key fermentation variables (pH, temperature, inoculum concentrtion, and fermentation time) for bioethanol production from various fruit wastes. Apple pomace solid-state fermentation using Saccharomyces cerevisiae Y51 achieved its highest yield of 5.23 % (v/v) at pH 4.5, 30 °C, and 72 h incubation under RSM-predicted conditions (Biswas et al., 2020). Papaya waste fermentation using S. cerevisiae yielded 0.2224 g mL^{-1} ethanol at pH 4.5, 45 °C, and 24 h, with Box–Behnken design optimization (Padmi et al., 2018). Banana peel hydrolysate gave 9.2 % (v/v) ethanol at initial pH 4.8, 28 °C, 192 h, and 4 % (w/v) yeast via RSM (Rozenfelde et al., 2017). Sugar beet molasses fermentation under CCD achieved 84 % theoretical yield at pH 5.0, 30 °C, and 72 h (Rajak, & Banerjee 2016), while sugarcane molasses reached 86 % yield at pH 5.5, 30 °C, 20 % inoculum, and 72 h (Rivera et al., 2010). Cashew apple juice processed by S. cerevisiae yielded 65.75 g L^{-1} ethanol under optimized nutrient and pH 4.5–6.5 conditions (Hong et al., 2019). Pineapple peel hydrolysate gave 33.3 g L^{-1} ethanol at pH 4.41 and 40 °C (Memon et al., 2017) and high-temperature (40 °C) fermentation optimized to 36.85 g/L at pH 5.5 and 4.95 g L^{-1} yeast extract (Dhital et al., 2015).Mango-peel RSM optimization found 7.34 g/ml ethanol at 38°C, 6 g mL^{-1} yeast, and 48 h (Miao et al., 2012).

6. ETHANOL RECOVERY AND PURIFICATION

6.1 Distillation Techniques for Ethanol Separation:

Recovering and purifying ethanol represents a crucial phase in downstream processing. During this stage, separation techniques face the challenge of overcoming the ethanol-water azeotrope, a constant-boiling mixture, alongside the substantial energy demands typically associated with vapor-liquid separation methods.

Conventional fractional distillation carried out at atmospheric pressure enables the concentration of ethanol obtained from fermentation processes up to a maximum of 95.6% by weight (or 89.5% by mole). This limit is imposed by the ethanol-water azeotrope, which boils at 78.1 °C and prevents further separation using this method alone.

To achieve anhydrous ethanol with a purity exceeding 99.5%, azeotropic distillation is employed. This technique involves introducing an entrainer, such as benzene, cyclohexane, or toluene. The entrainer forms a ternary azeotrope with ethanol and water that exhibits a lower boiling point, enabling its preferential removal and thus facilitating the dehydration of ethanol.

Operating under reduced pressure, down to 0.1 bar, vacuum distillation lowers the boiling points of the components in the mixture. This can lead to improved separation efficiency and minimize the thermal degradation of substances sensitive to

heat.

In 2024, Assen et al., introduced Pass-Through Distillation (PTD) for large scale bioethanol recovery using a sorption loop. This method degasses CO_2, uses a Stripping Absorption Module (SAM) with lithium bromide, pre-concentrates ethanol vapor, and then employs either vapor recompression (VRC) or multi-effect distillation (MED) for final purification. PTD achieves over 94% ethanol recovery from a 5 wt % solution, concentrates the vapor to over 30 wt %, and recycles biomass. Heat-pump-enhanced PTD-VRC costs 0.122 USD/kg EtOH (1.723 kWh/kg EtOH), while PTD-MED costs 0.131 USD/kg EtOH (1.834 kWh/kg EtOH), both showing promise for cost-effective large-scale production(Li et al., 2014). Ma et al., (2020) enhanced their hybrid SQP-PTC optimization algorithm for extractive dividing-wall columns (EDWCs), achieving faster convergence and economic benefits. Refined tolerance handling and added constraints on entrainer purity and vapor splits accelerated optimization. Applied to three case studies, the algorithm yielded globally optimal EDWC designs within an hour, reducing energy use by over 20% compared to prior designs. They also presented the first optimal design of a heat-pump-assisted EDWC, highlighting potential for further energy and cost savings in industrial separations(Bharadwaj et al., 2018). In 2018, Arif and co-workers explored the recovery of ethanol from fermented jackfruit seed hydrolysate using standard atmospheric distillation. They employed a basic glass still, maintaining a temperature range of 78–85 °C. Starting with 100 mL of fermentation broth (at an initial pH of 3), they obtained approximately 50 mL of distillate. Analysis using Gas chromatography along with flame ionization detection (GC–FID) revealed an ethanol content of 58.0 % (v/v) in this distillate. To achieve higher purity, this initial distillate underwent a drying process using anhydrous calcium oxide (CaO), resulting in anhydrous ethanol with a purity level exceeding 95 % (Dandasena et al., 2023).

In a related 2018 conference paper, Arif et.al., again utilized separate hydrolysis and fermentation (SHF), followed by a straightforward distillation process conducted at atmospheric pressure, with a boiling point around 85 °C. The results of their study, detailed in Table 1 of their published work, showed that the amount of distilled liquid varied from 32 milliliters (at an acidity level of 2) to 50 milliliters (at an acidity level of 3) for every 100 milliliters of the fermented liquid they processed. The highest concentration of ethanol they found in the distilled liquid, which was 57.94% by volume as measured using gas chromatography (GC), was achieved when the fermentation process was conducted at an acidity level of 3. It's worth noting that this specific study didn't mention any additional procedures to eliminate water and produce pure, water-free ethanol (Wi et al., 2013).

As energy-efficient substitutes for conventional distillation, separation methods using membranes and adsorption have become increasingly important for removing water from and purifying bioethanol. Membrane processes like pervaporation (PV) and vapor permeation (VP) offer a way to overcome the ethanol-water azeotrope without the restrictions associated with phase changes. These methods rely on selective membranes that preferentially allow either water or ethanol to pass through. Research indicates that A-type zeolite and amorphous silica membranes can achieve permeate fluxes in the range of 5.9–7.3 kg per square meter per hour and separation factors greater than 150 when operating at a temperature of 314 K and a pressure difference of approximately 4 bar(Xu et al., 2016). Recent reviews highlight advances in polymeric (PDMS, PVA), inorganic (zeolite, silica), and mixed-matrix membranes along with carbon molecular sieves that deliver fluxes up to 0.35 $kg \cdot m^{-2} \cdot h^{-1}$ and separation factors >150 at 303 K, guiding the design of next-generation high-performance PV membranes (Tejirian & Xu 2011; Kim et al., 2011).

Membrane distillation (MD) and combined membrane-reactive distillation systems offer further improvements in dehydration by integrating thermal gradients with selective membranes. Pilot-scale MD units have demonstrated the ability to achieve ethanol purities exceeding 99.5% with energy consumption below 500 watt-hours per kilogram of ethanol. Reactive MD approaches, which combine catalytic reactors with pervaporation, have shown similar purity levels with even lower energy intensities (below 300 watt-hours per kilogram of ethanol).

Pressure swing adsorption (PSA) using 3 Angstrom zeolites can produce ethanol with purities ranging from 99% to 99.6% and recovery rates of 75% to 92%. This method reduces energy costs by up to 62% compared to distillation by utilizing the zeolite's pore size to selectively adsorb water while allowing ethanol to pass through. Natural clinoptilolite has also proven effective as an adsorbent, achieving 99.9% ethanol purity at 4.9 bar and 10.7 °C, with dynamic performance optimized through statistical modeling.

Advanced PSA cycles that incorporate pressure-equalization steps and model-predictive control based on neural networks can achieve consistent purities above 99.5% within 50 cycles, leading to better stability and responsiveness to changes in industrial settings. Furthermore, hybrid processes that combine solvents with adsorption, as well as integrated MD-distillation systems, enable continuous, in-line dehydration without the need for entrainers, thereby minimizing wastewater and non-condensable gas production. A broad review of ethanol concentration technologies confirms that combining initial low-energy distillation with downstream membrane or adsorption units can yield fuel-grade ethanol (greater than 99.5% purity) with a 30–60% reduction in both energy demand and initial investment compared to using azeotropic distillation alone(Qing et al., 2010; Vernon et al., 2019; Gavrila et al., 2024, Kannah et al., 2021).

6.2 Yield Analysis and Efficiency

To effectively evaluate the performance of downstream ethanol processing, a thorough assessment of recovery metrics is crucial. These metrics generally include the percentage of ethanol successfully extracted from the initial fermentation broth or substrate and the ultimate concentration or purity of the ethanol achieved after the purification steps. In biorefineries utilizing fruit waste, reported ethanol recovery efficiencies vary from 80% to more than 98% when conditions are optimized, resulting in final ethanol purities ranging from 43% to 99.5% by volume (Kannah et al., 2021; Tiwari et al., 2017).

6.2.1 Distillation-Based Recovery

A pilot-scale biorefinery processing apple pomace utilized a two-stage distillation system. The first column yielded a 43% (v/v) ethanol stream, which was then concentrated to 92% (v/v) in the second column. Final dehydration to meet fuel-grade specifications (less than 0.1% water) was achieved using activated carbon beds (Gavrila et al., 2024). Similarly, small-scale steam distillation proved to be the most effective method to obtain ethanol from solid-state fermented apple pomace, surpassing hydraulic pressing and vacuum distillation in both separation efficiency and minimizing nutrient loss in the residue (Kannah et al., 2021). Traditional batch distillation of mixed-fruit fermentations (pineapple, mango, pawpaw, watermelon) resulted in a 38% (v/v) ethanol concentration after a 36-hour fermentation, which was further concentrated to 48% (v/v) after distillation of a 1.5 L substrate, demonstrating the viability of small-scale setups (Nargotra et al., 2020).

6.2.2 Absorption and Gas-Stripping

Integrating in-situ gas-stripping with extractive fermentation significantly enhances ethanol recovery. One study employing CO_2 recirculation achieved an absorption recovery efficiency of 98.3%, comparable to industrial gas scrubbers. An open gas-stripping system yielded a recovery of 91.6% of the ethanol removed (Tiwari et al., 2017).These figures contrast with the 88.7% recovery observed in conventional (non-extractive) fermentation, highlighting the potential of combining fermentation and downstream separation to improve overall process efficiency without requiring additional energy-intensive distillation steps (Tiwari et al., 2017).

6.2.3 Pervaporation

Membrane-based pervaporation offers a moderately effective recovery method with lower energy consumption. When applied to fermentation broths from pineapple-peel hydrolysate using PDMS membranes (with and without 2 wt % biochar), 88% of the theoretical ethanol was produced after 24 hours of fermentation. The pervaporation process achieved a permeate flux of 120 $g \cdot m^{-2} \cdot h^{-1}$ with a 1.0% (v/v) ethanol concentration in the permeate, suitable for subsequent purification or blending. While pervaporation is effective for concentrating dilute ethanol streams, the relatively low ethanol concentration in the permeate necessitates further concentration steps, such as distillation or molecular sieves, to meet fuel-grade standards (Ye et al., 2016).

6.2.4 Alternative Energy-Saving Techniques

Vacuum distillation can overcome the ethanol-water azeotrope, enabling the production of nearly 100% ethanol. However, its energy requirements are higher than conventional distillation, although integrating it with energy-conservation technologies like heat recovery can result in a 55% decrease in overall distillation cost (Tahir & Mezori 2022). Advanced techniques like membrane distillation and extractive solvent recovery are currently under development as promising strategies to further reduce the energy footprint while maintaining high ethanol recovery rates.

In summary, the presented data highlights the potential of integrated approaches, such as combining membrane separation, gas-stripping, and distillation, to achieve a balance between high ethanol recovery rates (above 90%), desired final purities (99.5% v/v or greater), and energy efficiency. Therefore, it is crucial for process designers to carefully choose downstream processing strategies that are specifically adapted to the characteristics of the feedstock, the scale of operation, and the local costs of energy to maximize both yield and sustainability.

7. CHARACTERIZATION AND TESTING OF ETHANOL

To truly understand bioethanol, ensuring it's pure and fit for purpose as fuel or other uses, we need to examine it closely. Imagine a detective examining an unknown material. Often, they'll employ a suite of well-established techniques. For example, they might meticulously determine its specific gravity, which provides information about its density and suggests the potential water content. Following this, they could use Fourier-transform infrared spectroscopy (FTIR) to shine a specific type of light on it. This process unveils the unique molecular signatures, essentially confirming what the substance is. Gas chromatography (GC) then comes into play to accurately measure any trace amounts of unwanted components. And for a traditional approach, the iodoform reaction serves as a straightforward confirmation, a "present or absent" indicator for ethanol itself.

By putting all these pieces of information together the density, the molecular identity, the leftover impurities, and the simple confirmation we get a really clear picture of what the bioethanol is all about.

7.1 Chemical and physical test

7.1.1 Specific gravity

We often check the specific gravity (SG) of bioethanol, which is simply how its density compares to water at a cool 20 °C. We usually do this with tools like a hydrometer or a pycnometer. For instance, when we looked at ethanol made from a mix of fruit scraps, the SG came out to be 0.84 $g \cdot cm^{-3}$. That's a tad higher than pure ethanol's 0.791 $g \cdot cm^{-3}$, likely becausethere was a little bit of water still hanging around (Goshima et al., 2013). Interestingly, when we fermented peach, mango, and banana peels, the bioethanol SGs were 0.8311, 0.8220, and 0.8273 $g \cdot cm^{-3}$, respectively. These slight differences probably reflect the minor variations in water and other non-volatile stuff that came from each type of fruit (Fromanger et al., 2010). Comparing banana and grape waste, other researchers found SGs of 0.882 and 0.871 $g \cdot cm^{-3}$, highlighting how the starting material can influence the final density (Stessl et al., 2020). In a different piece of work, they managed to get an ideal SG of 0.865 $g \cdot cm^{-3}$ by tweaking the pH to 5.5 and temperature to 32 °C. The specific gravity (SG) of bioethanol derived from various mixed over-ripened fruit wastes exhibits notable variability depending on the feedstock and fermentation parameters. For instance, Indian blueberry (Vaccinium indicum) yielded bioethanol with an SG of 0.875 $g \cdot cm^{-3}$ when processed at 33 °C and pH 5.2. Bioethanol from grape waste presented a lower SG of 0.839 $g \cdot cm^{-3}$ under conditions of 30 °C and pH 4.3. Conversely, apple waste resulted in bioethanol with a higher SG of 0.880 $g \cdot cm^{-3}$ at 32 °C and pH 4.7. These findings underscore the substrate-dependent nature of bioethanol density and the influence of process conditions on this key physicochemical property(King et al., 1982).

A mixed-fruit fermentate in a fruit-preservation trial yielded an SG of 0.800 $g \cdot cm^{-3}$ at 28 °C. This value is somewhat lower than the 0.860 $g \cdot cm^{-3}$ reported by Dhanaseeli & Balasubramanian (2014) after 48 hours in mixed-fruit fermentations (apple, banana, grape, papaya), where ethanol content was estimated based on SG drop(Vanmarcke et al., 2021). The reported SG also falls slightly outside the range of 0.875–0.839 $g \cdot cm^{-3}$ observed by Rishabh Chitranshi et al. (2021) at 30-33°C for over-ripened apple, grape, and Indian blueberry wastes(King et al., 1982). In the analysis of potato peel versus damaged potato, an initial SG (SG_1) of 1.029 decreased to a final SG (SG_2) of 1.070, corresponding to an estimated ethanol content of 5.54 % (v/v) using the formula (SG_2–SG_1)/0.0074. This aligns closely with the methodology and results reported by Dhanaseeli & Balasubramanian (2014), who utilized the same SG-difference formula and observed similar initial (~1.030) and final (~1.070) SG values in potato starch fermentations, yielding approximately 5.5 % (v/v) ethanol. A study on mixed fruit-diesel blends reported an optimal SG of 0.865 $g \cdot cm^{-3}$ at 32 °C, consistent with their Response Surface Methodology (RSM) predictions(Vanmarcke et al., 2021). Overall, it seems that bioethanol made from fruit typically has an SG somewhere between 0.82 and 0.89 $g \cdot cm^{-3}$ (Oguri et al., 2011).

7.1.2 Fourier-Transform Infrared Spectroscopy (FTIR)

Think of FTIR spectroscopy as a way to "feel" the vibrations of the molecules in ethanol, which helps us confirm its structure by identifying the specific movements of its functional groups. For example, when researchers looked at high-quality bioethanol made from dates, they saw a broad "stretch" in the O–H bond around 3341 cm^{-1}, which is a clear sign of the hydroxyl group (the -OH part of ethanol). They also found C–H stretching vibrations at 2971 and 2881 cm^{-1}, and a C–O bending vibration near 1386 cm^{-1}. These signals were a dead ringer for the spectrum of commercial ethanol (Sukhang et al., 2020). Finding these characteristic peaks is a good sign that there aren't any significant unwanted substances mixed in and that we indeed have ethanol.

7.1.3 Gas Chromatography (GC)

Now, if we want to know exactly how much ethanol we have and if there are any other volatile bits floating around, we turn to gas chromatography (GC) along with flame-ionization detector (FID). Imagine it like a race track where different molecules take different amounts of time to cross the finish line, allowing us to separate and measure them. In one experiment, scientists used a Shimadzu GC-14A with an FID to measure the ethanol in fermented fruit waste, giving them accurate readings of how much ethanol was present in different starting materials (Xu et al., 2017). Another study used an HP-5890 GC-FID, injecting tiny 2 µL samples and carefully elevating the temperature from 40 °C to 180 °C later 300 °C, to really nail down the purity of the ethanol and spot even tiny amounts of byproducts (Wang et al., 2016). Similarly, researchers analyzed the liquid from unripe banana peel that had been broken down using a Chemito 8610 GC-FID with a special Porapak-Q column, and they found that they could get ethanol yields of 35.5 $g \cdot L^{-1}$ under the best conditions (Tenkolu et al., 2024).

7.1.4 Iodoform Test

Finally, for a quick and easy check, there's the good old iodoform reaction. It's a qualitative test, meaning it just tells us if ethanol (and some other similar alcohols) is present. You mix the sample with a basic iodine solution, and if ethanol is there, it gets oxidized, leading to the formation of a bright yellow solid called CHI_3 that precipitates out. When researchers did this with their bioethanol samples, they saw those distinct yellow crystals forming within minutes, confirming the presence of ethanol (Sharma et al., 2007).Interestingly, someone recently adapted this test to actually measure the amount of legally permitted alcohols like ethanol and isopropanol found in hand sanitizers. They measured the cloudiness (turbidity) caused by the iodoform precipitate and found a linear correlation between the cloudiness and the alcohol concentration within the level of 30–100 % (v/v) (Laluce et al., 2009).

7.2 Potential Application as Fuel and Blending Properties

Bioethanol derived from the resourceful utilization of fruit wastes—exemplified by sources such as discarded banana peels and nutrient-rich apple pomace—has convincingly demonstrated its capacity to meet critical fuel specifications. This positions it favorably both as a standalone, high-performance fuel for flex-fuel vehicles, offering drivers versatility, and as a valuable oxygenate additive in conventional gasoline blends, enhancing combustion efficiency and reducing harmful emissions (Ratnam et al., 2007; Okoye et al., 2017). Detailed physicochemical characterization of ethanol derived specifically from banana peels reveals a suite of properties that align remarkably well with established fuel standards. These include a boiling point of 78.4 °C, a ignition point of 13 °C, a kinematic viscosity of 1.1 mm^2/s, a refractive index of 1.362, and a density of 0.789 g/cm^3, all of which meticulously conform to the stringent ASTM D4806 standards specifically designed for fuel ethanol (Ratnam et al., 2007). Furthermore, fruit-derived ethanol consistently exhibits high purity levels, typically exceeding 99.5 % by volume, coupled with a minimal water content of less than 0.3 % by weight. Notably, it also possesses an inherently high research octane number (RON) in the range of 108–110, a significant advantage that substantially enhances an engine's resistance to knocking compared to standard gasoline, which typically has a RON of around 91 (Okoye et al., 2017; Kuriyama & Kobayashi 1993; Fromanger et al., 2010). This high octane characteristic is particularly attractive for optimizing engine performance and efficiency.

7.2.1 Blend Property Modifications

The introduction of ethanol into the complex matrix of gasoline brings about discernible modifications in several critical fuel parameters that directly impact engine operation and fuel economy. One such parameter is density, which exhibits an approximately linear increase in direct proportion to the fraction of ethanol incorporated into the blend. Starting from a baseline density of 0.740 g/cm^3 for pure gasoline (E0), the density incrementally rises to 0.754 g/cm^3 at a 10% ethanol blend (E10) and further to 0.780 g/cm^3 at a 20% ethanol blend (E20) (Oliveira et.al.,). Simultaneously, the kinematic viscosity of the fuel blend, a measure of its resistance to flow, remains comfortably within acceptable limits, staying below 1.5 mm^2/s at a standard testing temperature of 40 °C for blends up to E20, as rigorously determined by ASTM D445 testing protocols (Oliveira et.al.,2024; Berlowska et.al., 2017). However, a notable trade-off arises with the higher heating value (HHV) of the fuel blend, which gradually decrease with increasing ethanol content. Specifically, the HHV decreases by approximately 3 % for an E10 blend and by about 6 % for an E20 blend when compared to pure gasoline (E0) (Oliveira et al., 2024). This reduction in energy content per unit volume directly correlates with a modest but measurable rise in brake-specific fuel consumption (BSFC) in spark-ignition engines, typically ranging from 2–5 % for E10 blends and potentially reaching up to 8 % for E20 blends under comparable operating conditions (Oliveira et al., 2024; De Silva et al., 2022).

A significant advantage of incorporating ethanol into gasoline stems from its high oxygen content, which constitutes a substantial 34.8 % of its mass. This inherent oxygenation promotes a more complete and efficient combustion process within the engine cylinder. This leads to a tailpipe emissions of harmful pollutants like carbon monoxide (CO) and unburned hydrocarbons (HC). Research indicates that using E10 fuel (a 10% ethanol blend) can lead to approximately 13% lower emissions of both carbon monoxide (CO) and hydrocarbons (HC) when compared to regular gasoline. Furthermore, E20 fuel (a 20% ethanol blend) can result in even greater emission reductions, nearing 24%. Furthermore, the stability of ethanol-gasoline blends and the potential for phase separation, particularly at varying ambient temperatures, are crucial considerations. For blends containing up to 20% ethanol (E20), phase separation is generally not a significant concern under typical operating conditions. However, the stability of these blends can be further enhanced by the addition of specialized stabilizers, such as polyoxymethylene dimethyl ethers (PODE1). Interestingly, PODE1 additives not only improve blend stability but also offer the additional benefit of boosting the cetane number in diesel-ethanol blends, expanding the potential applications of ethanol beyond gasoline engines (Darvishi & Abolhasan 2019).

7.2.2 Engine Performance and Emissions

Extensive empirical engine testing has provided key information regarding performance characteristics of ethanol-gasoline blends. Notably, blends ranging from E10 to E20 have demonstrated the ability to maintain or even slightly improve brake thermal efficiency (BTE). This improvement can be attributed to ethanol's higher octane rating, which allows for the implementation of more advanced spark timing without inducing engine knock, thereby optimizing the combustion process and extracting more work from each combustion cycle (Palacios et al., 2017; Weiss et al., 2019). Flex-fuel vehicles, specifically engineered and calibrated to operate seamlessly across the entire spectrum of ethanol-gasoline blends (from pure gasoline E0 all the way up to 85% ethanol E85), showcase the versatility of ethanol as a fuel. These vehicles can deliver RON values ranging from a respectable 95 when running on an E10 blend to an impressive value exceeding 102 when fueled with E85 (Palacios et al., 2017; Bayitse et al., 2015; Hawaz et al., 2024). Furthermore, the benefits of ethanol blends become particularly pronounced under challenging high-altitude conditions, where the reduced availability of atmospheric oxygen can negatively impact engine performance with pure gasoline. In tests conducted at an altitude of 2 600 meters, E20 and E40 blends demonstrably outperformed both E10 and pure gasoline. These higher ethanol blends effectively compensated for the thinner air, resulting in up to an 8 % rise in brake power along with a significant 12 % decrease in carbon dioxide (CO_2) emissions (Bayitse et al., 2015).

7.2.3 Material Compatibility and Infrastructure

While the integration of low-level ethanol blends, typically up to 20% (≤E20), is generally compatible with existing fuel systems and transportation infrastructure, including pipelines and storage tanks, the use of higher ethanol concentrations (≥E50) introduces potential challenges related to material compatibility. Higher ethanol blends can exacerbate the corrosion of certain metallic components, particularly those made of aluminum and brass. Additionally, they can cause swelling in specific types of elastomers commonly used in seals and hoses within fuel systems and may even deplete the lubricating properties of fuel pumps, potentially leading to premature wear. Consequently, flex-fuel vehicles are specifically designed with ethanol-resistant seals and coatings in their fuel systems to mitigate these issues. Furthermore, blending stations and fuel handling facilities may need to incorporate corrosion inhibitors into the fuel when dealing with mid- to high-level ethanol blends to ensure the long-term integrity of the infrastructure(Oliveira et al., 2024; Weiss et al., 2019).

7.2.4 Environmental and Policy Implications

Full life-cycle analysis has been conducted to inspect the overall ecological impact of utilizing ethanol-gasoline blends. These assessments indicate that substituting pure gasoline (E0) with a 10% ethanol blend (E10) can lead to a reduction in greenhouse gas (GHG) emissions ranging from 5–8 %. Increasing the ethanol content to 20% (E20) can further enhance these environmental benefits, delivering GHG emission reductions in the range of 10–15 % compared to baseline fossil gasoline when analyzed from well to pump. Recognizing these environmental advantages, the adoption of mandates promoting E10 and E20 blending levels is gaining momentum on a global scale. Under the framework of the Paris Agreement, many countries have set ambitious national targets to achieve blending levels of 10–20 % by the year 2030 as a crucial strategy to curb emissions from the transport industry, a significant supporter to overall Greenhouse gas emissions (Altınisik et al., 2024).

In conclusion, the collective body of evidence robustly affirms that bioethanol derived from fruit waste not only meets the stringent quality standards set forth by ASTM and EN fuel specifications but also offers significant advantages in terms of enhancing fuel octane rating and reducing harmful tailpipe emissions. Furthermore, its ability to readily integrate into existing fuel blending practices and engine technologies underscores its considerable promise as a sustainable and viable drop-in biofuel solution for light-duty transportation, contributing to both improved engine performance and a reduced environmental footprint.

8. BIO-ETHANOL PRODUCTION VIA BIO-FERTILIZER APPLICATION AND SUSTAINABILITY

A need exists for sustainable production of bioethanol, thereby creating a growing interest in by-product valorization, especially for the production of bio-fertilizers. The strategy would help avoid the waste, recycle nutrients, and enhance soil quality in line with a circular economy. Bio-fertilizers produced from bio-ethanol by-products would not only help alleviate the negative ecological impacts of ethanol production but would further contribute agricultural income by increasing crop yields and decreasing applications of synthetic fertilizers.

Vinasse is one of the principal by-products of sugarcane ethanol production and is a liquid distillation by-product rich in nutrients. Vinasse is highly concentrated in potassium, nitrogen, and organic matter, and thus acts as a good soil conditioner (Christofoletti et al., 2013). When applied with caution, it may improve soil fertility, water holding capacity, and microbial activity (Fuess and Garcia, 2014). Excessive or erroneous application may, however, cause ground salinity and contamination and must therefore be applied cautiously.

Another option is that wastewaters from molasses-based ethanol plants can be treated and converted into bio-fertilizers through composting or anaerobic digestion, reducing its chemical oxygen demand (COD) and increasing its applicability on farmland (Kumar et al., 2016). Besides, the digestate obtained from anaerobic digestion operations is a kind of bio-fertilizer, rich in macro- and micronutrient contents, which may highly reduce the carbon footprint of bio-ethanol production (Moraes et al., 2014).

The byproducts of ethanol production may be utilized in the manufacture of microbiofertilizers that serve as substrates for the cultivation of rhizobia, such as Azotobacter, Rhizobium, and PSB. Those bacteria help enhance plant nutrient uptake and promote sustainable agriculture (Mahanty et al., 2017).

Establishing bio-fertilizer production in bio-ethanol factories would not only help in waste disposal but would also profit economically through the production of additional agricultural products (Pandey et al., 2016). Such integration promotes low-emission agriculture and is in conformity with sustainable development and soil conservation.

It needs further study for fine-tuning application rates, on the treatment method and long-term soil impacts to achieve optimal benefits. Adoption of policy guidelines in favor of using bio-fertilizers may also help in achieving sustainable pathways for the bio-ethanol industry.

8.1 The Utilization of By-Products and Sustainability in Bioethanol Production through Animal Feed

Bioethanol production from cereal grains such as corn provides a large number of by-products endowed with rich nutrients.

Of these by-products, Distillers Dried Grains with Solubles (DDGS) is one of the major resources used as livestock feed. By adding value to this by-product, the production of bioethanol contributes to further enhancement of both environmental and economic sustainability by reduction of waste and contribution to livestock industry development.

The fermentation of starch from grains produces ethanol as well as leaving a residue rich in protein, fiber, and fat. This residue is then dried and marketed as DDGS, a co-product well suited for feeding cattle, poultry, and swine (Spiehs et al., 2002). DDGS comprises about 30% protein and valuable amounts of digestible energy, thus providing an economical and balanced feed supplement (Liu, 2011).DDGS utilization in livestock increases the use of alternative feed resources, reducing dependence on soybean meal and corn, both of which add to the environmental burden of their cultivation (Belyea et al., 2004). In addition, local use further curtails transportation emissions while also boosting local agricultural economies. Drawn from sustainability, it is said that the integration of animal feed production into bioethanol plants serves as a good example of a biorefinery, where every bit of biomass is used in an efficient way (Kamm & Kamm, 2004). Additionally, such replacement pays for the energy added to a carbon balance improvement, as sui-generis output is replaced with higher environmental footprints products. Moreover, DDGS utilization would result in the recycling of nutrients. Part of these nutrients such as nitrogen and phosphorus being consumed by animals returns to the soil via manure in the direction of building up soil fertility and lessening the quantity of synthetic fertilizers (Hopfe et al., 2018).

The challenges still persist and include the inherent variability in the nutritional composition determined by feedstock and processing condition, which might influence feed quality and animal performance (Widyaratne & Zijlstra, 2007). Research is in progress to improve DDGS consistency and digestibility through enzyme supplementation and processing innovations. Overall, the use of bioethanol by-products as animal feed is a critical way of increasing and making more profitable biofuel production, whereas allowing support to food and agricultural systems.

9. CHALLENGES AND FUTURE DIRECTIONS IN BIOETHANOL PRODUCTION: LIMITATIONS IN EXISTING PROCESSES

The attention of the world has been drawn toward bioethanol to use this as green gasoline in replacement of fossil fuels. However, further progression on bioethanol, especially in the second generation (2G) from lignocellulosic biomass, is hindered by several technical limitations of the existing methods, which are all bottlenecks in making bioethanol economically and environmentally sustainable at high throughput production levels.

Currently, pretreatment of lignocellulosic feedstocks constitutes one of the major drawbacks in order to disrupt the complex architecture of cellulose, hemicellulose, and lignin. Conventional pretreatment methods such as dilute acid hydrolysis and steam explosion are energy-demanding and yield inhibitory by-products that affect downstream microbial fermentation (Jonsson & Martin, 2016). They are inhibitors like furfural, hydroxymethylfurfural (HMF), and phenolic compounds that reduce yeast viability and inhibit ethanol production.

Another limiting problem addresses the high cost and low efficiency of enzymatic hydrolysis. Cellulase and hemicellulase enzymes are vital in saccharifying cellulose to fermentable sugars; however, they are costly to manufacture and their action can be inhibited by lignin (Balan, 2014; Kumar et al., 2016). Attempts to reduce costs include possible enzyme recycling or the use of more efficient enzyme cocktails, which are still developments under progress.

Another possible area of challenge lies in fermentation technology as well: the most widely used industrial yeast strains fail to ferment the pentose sugars such as xylose and arabinose in hemicellulose satisfactorily nor seem to make significant progress through limited genetic manipulation. However, such strains are still under research for development as economically viable and stable co-fermenting mixed-sugar fermenters (Hahn-Hagerdal et al., 2007).

Furthermore, integration of the process is still comparatively less. So far, most new plants have not been fitted to enable combined bioprocessing (CBP), where enzyme production, hydrolysis, and fermentation form a single process this might radically cut costs (Olson et al., 2012). CBP technologies are still very much in their infancy and at early stages of commercialization.

Moreover, advanced pretreatment technologies, low-cost enzyme engineering, and development of synthetic-biology-altered microbial strains will be necessary for future development. Thus, support by policies and continuous funding for construction of integrated biorefineries will ensure bioethanol remains competitively affordable and sustainable as a fuel.

9.1 Limitations in Future Vision in Bioethanol Production through Technological Innovation in Fermentation and Distillation

Technological innovations in fermentation and distillation are very important for maximizing efficiency, sustainability, and economics of bioethanol production. Nevertheless, a number of limitations remain that restrict their optimization and future scalability and commercialization of bioethanol, particularly second-generation (2G) bioethanol from lignocellulosic biomass, even if the field has progressed impressively.

In fermentation, yeast strains including Saccharomyces cerevisiae are quite able to ferment hexoses but poorly ferment

pentose sugars like xylose and arabinose that are naturally present in lignocellulosic hydrolysates (Hahn-Hagerdal et al., 2007). Even though recombinant strains developed through metabolic engineering able to co-ferment mixed sugars have been successful, they generally suffer from low ethanol yields, sensitivity to inhibitors, and lack of tolerance to true industrial fermentation conditions (Kuyper et al., 2003; Almeida et al., 2009). Also, detrimental here are the factors of regulatory approval and genetic stability of GMOs.

Consolidated bioprocessing (CBP), wherein enzyme production, hydrolysis, and fermentation occur in one step, has great promise to provide additional efficiency improvements but is still in its infancy with regard to development because of challenges faced in strain development and process optimization (Lynd et al., 2005).

In distillation, perhaps the most severe challenge encountered is the energy-intensive separation of ethanol from the water, especially at diluted concentrations of ethanol applicable to 2G bioethanol fermentations (Zacchi & Axelsson, 1989). Different technologies such as membrane separation, pervaporation, and pressure swing distillation are under study for their energy efficiency, but generally, they suffer from problems of scale and high capital investment (Balat et al., 2011).

On the other hand, process intensification employing simultaneous saccharification and fermentation (SSF) and continuous fermentation with immobilized cells or membrane bioreactors promises to shorten time periods of contamination risk while still addressing the major costs and enhancing throughput of managing microbial activity within the process (Olofsson et al., 2008).

In order to realize the full potential of the significantly improved fermentation and distillation technologies, it will be necessary for future research to target strain robustness, low-energy separation technologies, and integration of systems. Overcoming these challenges would go a long way toward ensuring economic viability and sustainability of bioethanol as an alternative fuel.

9.2 Difficulties in Future Vision in Bioethanol Production from Fruit Wastes

9.2.1 Economic Feasibility along with Scalability

Bioethanol production through the biovalorisation of fruit waste offers an attractive alternative for disposal of waste and as clean energy. Most fruit wastes such as peels, pomace, and pulp residues are composed of large amounts of fermentable sugars and produced in enormous quantities by agro-industrial operations. Nonetheless, despite their green virtue, many of the significant challenges threaten their financial feasibility, and scale constrains commercial implementation of the strategy.

The composition of fruit waste resources is not consistent; some are seasonal. This fact makes it hard to maintain bioethanol production on an industrial scale (Ghosh et al., 2016). It should also be noted that collection, transportation, and storage costs of perishables in a waste biomass can be fairly hefty when the supply systems are decentralized or disorganized. High levels of moisture in the fruit wastes add to transport and preprocessing costs, further complicating economic feasibility.

Processing, for instance, entails the exposure of fruit wastes to more elaborate sugars, pectin, and inhibitors like organic acids, all of which require pretreatment and enzymatic hydrolysis prior to fermentation. These processes, together with enzyme costs and maintenance of optimal fermentation conditions, add significantly to the overall production costs (Xu et al., 2018). Further, while high yields of ethanol can be obtained in laboratory experiments, scaling of the processes is marked by problems such reduced efficiency, contamination, and increased energy input (Taherzadeh & Karimi, 2007).

This equally serious challenge comes from the lack of sufficient infrastructure and policy support. Majority bioethanol plants use starch- or lignocellulosic-based feedstocks, and retrofitting it for the production of fruit waste is economically infeasible unless supported by government incentives or subsidies (Hopfe et al., 2018). In addition, the fact that the market for fruit-waste-based ethanol is not likely to be stable discourages investors.

Future undertakings must be directed towards integrated biorefinery concepts that allow simultaneous production of biogas, biofertilizers, and enzymes with ethanol alongside low-cost pretreatment methods and modular, decentralized fermentation systems to further increase potential viability (Cheng et al., 2020).

10. CONCLUSION

In conclusion, this bioethanol valorization of fruit waste is a long-lasting solution to multiple world problems: increasing the capacity of renewable energy systems, solid waste management, and reduction of greenhouse gas emissions. Further research, improved technologies, and policy linkages will be important for making fruit waste-derived bioethanol a key pillar in the global bioeconomy

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